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Award Number: DAMD17-03-1-0110

TITLE: Optimization and Characterization of Prostate Cancer Targeting Peptides

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REPORT DATE: February 2005

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2005	Annual Summary (1 Feb 2003 - 31 Jan 2005)

4. TITLE AND SUBTITLE
Optimization and Characterization of Prostate Cancer Targeting Peptides

5. FUNDING NUMBERS
DAMD17-03-1-0110

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The goal of the project was to characterize and optimize previously identified peptide ligands targeting receptors expressed on the surface of DU-145H prostate cancer cell line. We performed structure activity study with these peptides and we identified the minimum required sequence for binding as well as the amino acids essential for the interaction with the receptor. The peptide lead structures were used to prepare several secondary libraries containing mostly unnatural building blocks in order to identify ligands with stronger affinity to the DU-145H. The lead peptides were also used to prepare the multimeric ligands and ligands for affinity labeling of cell surface receptors involved in the interaction. The results showed that these peptides can isolate alpha6 integrin from the cell lysate.

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

In this project, we plan to characterize and further optimize previously identified peptide ligands for cell surface receptors expressed on the DU-145H prostate carcinoma cell line. The optimized ligands will be prepared with a affinity label so that they can be used to isolate and identify the target protein receptor on the prostate cancer cell surface.

Body

During the first year of funding period, the "one-bead one-compound" combinatorial libraries proposed in **Task 1a** were synthesized. The libraries contain 3,111,696 compounds each and the density of the ligand on the exposed surface is fully substituted (100%) for the first library and 20% of maximal solid support substitution for the second library. The reason for "20% down-substituted" library synthesis is to increase the stringency of screening so that low affinity binding ligands will not bind to the cancer cells, but the high affinity ligands will. The general structure of the libraries is $cX^1GX^2GX^3X^4c$, wherein X^1 = 42 natural and unnatural amino acids bearing active hydrogen capable to participate in hydrogen bond interaction, and X^2, X^3 and X^4 were 42 bulky and hydrophobic unnatural amino acids. These building blocks were chosen according to the consensus motifs discovered for some epithelial cancers including a prostate cancer line. Based on the NMR secondary structure study and molecular modeling experiments, we have determined that these cyclic octapeptides (cXGXGXXc motif) have similar conformation with the β-turn in N-terminal part of the molecule involving the first four amino acids cX^1GX^2.

Both cXGXGXXc libraries were evaluated for binding to DU-145H, LNCap, PC3 prostate carcinoma cell **(Task 1b)** as well other cancer cell lines such as ovarian, lung and pancreatic cancer cell lines **(Task 4a)**. Several high affinity ligand were identified for ovarian cancer cell lines ES-2 and SKOV-3, but no high affinity ligands for prostate cancer cell lines were identified. We therefore have decided to use our previously discovered peptide ligand (kikmviswkg) for DU-145H as the lead compound to further develop the prostate cancer targeting ligand. First, the biotin labeled D-amino acid decapaptide kikmviswkg was tested using flow cytometry with normal prostate cell lines PrEC and PrSC as well as the DU-145H to confirm the specificity for the carcinoma cell line. In order to help us to design an appropriate focused library based on this ligand, we performed an "alanine walk" experiment, in which each residue of the parent peptide was replaced with a D-alanine, one at a time **(Task 3a)**. In addition, we have also performed truncated studies so that amino acids at both amino and carboxyl end were removed one at a time. These peptide analogs were synthesized in a biotinylated form, tethered to the carboxyl end through a hydrophilic linker. The relative binding affinities of these peptide analogs to DU-145H cells were evaluated by flow cytometry **(Task 3b)** using flourescently labeled streptavidin as a secondary reagent.

In the second year of the funding, the alanine walk experiment results preformed on decapeptide kikmviswkg were not found reproducible, however, the results of experiments with truncated peptides were highly reproducible. We found that the minumun required sequence with activity comparable to the parent decapaptide is the heptapeptide ikmvisw **(Task 3)**. The activity of the minimum sequence peptide was confirmed by adhession assay at various concentrations of peptide immobilized on the straptavidin coated surface. Fig 1. In the next study, we carried out the alanine walk experiment with the heptatpeptide ikmvisw. The results revealed (Fig 2) the importance of amino acids in positions 2,3,4,5 and 7. If comapred with the parent peptide kikmviswkg and with the sequence kmviywkag, discovered at the same time as the parent peptide, the requirements for the activity of the peptide were corroborated: The _kmvi_w is the minimum essential sequence for the biological activity **(Task 3)**.

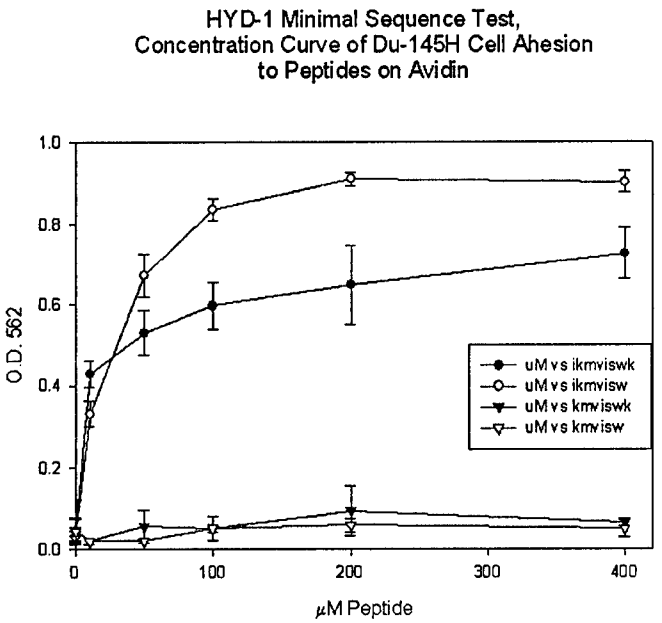

Fig 1: Adhesion assay of the DU-145H cells to peptide coated surface

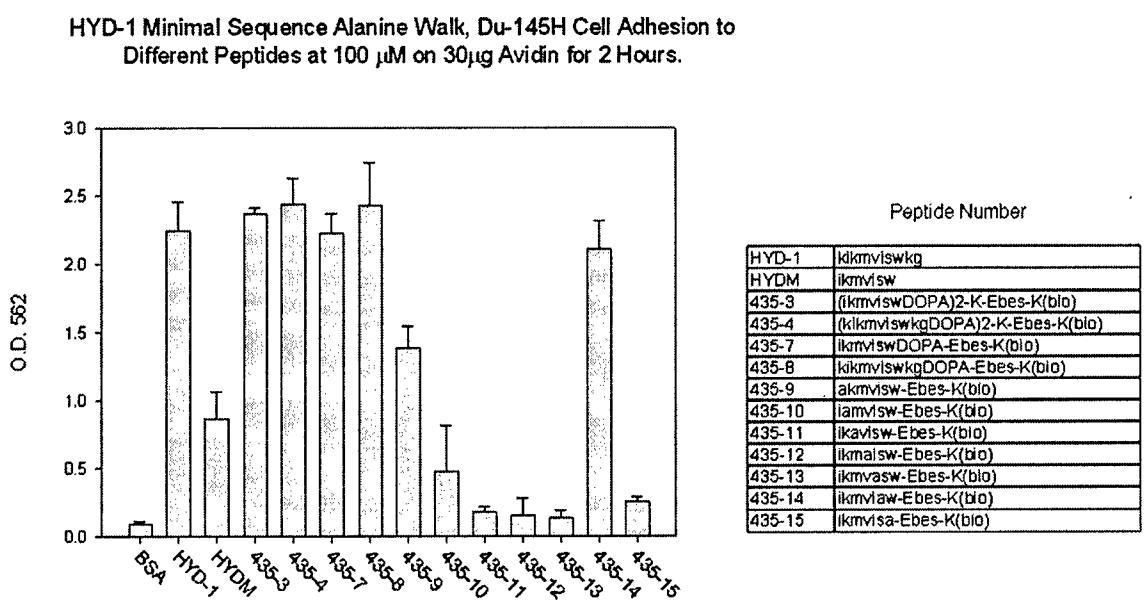

Fig 2: Ala walk on minimum sequence (entry 9-15) and activity of multimeric peptide ligands (entry 3-8).

We used the results of the above described structure activity study to design two secondary libraries based on the _kmvi_w motif **(Task 1a)**. Amino acids in positions 1 and 6 of the minimum sequence peptide were replaced with 42 unnatural D and L amino acids in two libraries. The first library was prepared with the bead surface substitution 50% of the original bead loading, and the remaining 50% was blocked with by acetylation. The second library was prepared with the surface substitution 20% only. The libraries are currently screened for DU-145H cell binding and the sequence on the active beads will be determined by microsequencing.

The minimum sequence heptapeptide ikmvisw and the parent decapaptide kikmviswkg were used for preparation of the dimeric ligands (Fig 3B) in order to further improve their biological properties **(Task 2)**. In general, multimeric ligands can show increased activity and improved *in-vivo* stability. The peptides were separated from the L-lysine, which serves as a branching unit, using hydrophilic Ebes linker (Fig 3) developed in our laboratory [2]. C-terminal part contained d-biotin for immobilization and conjugation during the biological experiments. The activity of the miltimeric ligands was tested and compared to the activity of the parent monomeric decapeptide and heptapeptide. We found that the activities are identical and the dimerization of the targeting peptide did not result in better activity (Fig 2). Although we did not observed any significant improvement, the dimeric ligands could have more convenient *in-vivo* pharmaco-kinetical properties than the parent monomers.

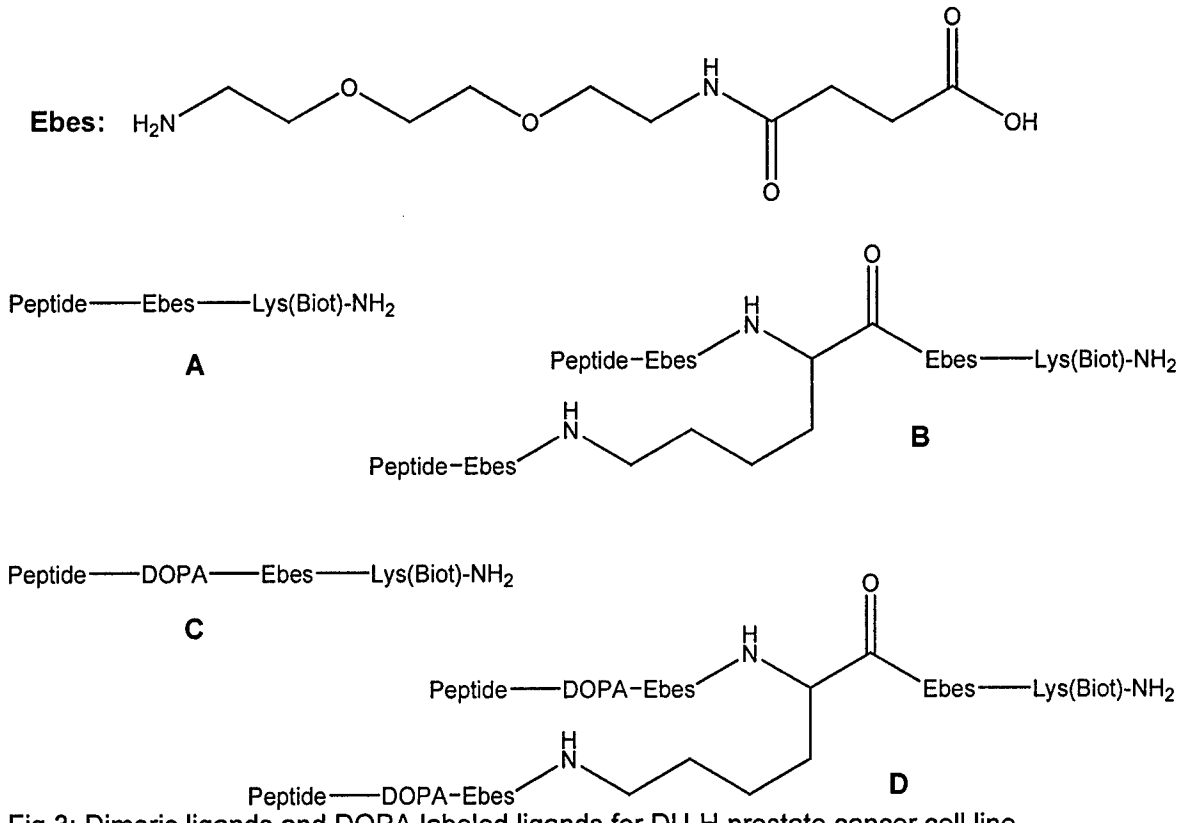

Fig 3: Dimeric ligands and DOPA labeled ligands for DU-H prostate cancer cell line.

The truncated peptides were used to isolate a putative receptor alpha6 **(Task 5)**. The peptide ligands were conjugated to the streptavidin coated beads and incubated with the DU-145H cell lysate. Then the beads were separated, washed and the isolated products were analyzed using Western blot (Fig 4). For the more efficient isolation of the receptor **(Task 5)**, further purification and analysis, covalent cross-linking of the targeting peptide decorated with fluorescent probe or biotin is necessary. Recently, we had more success with periodate triggered chemical cross-linking [3] rather then photoaffinity cross-linking methodology. Therefore we prepared the monomeric (Fig 3C) and dimeric (Fig 3D) analogs of targeting peptides with dihydroxyphenylalanine (DOPA) attached at the C-terminal part of the peptide. The activity of the DOPA modified peptides was tested and compared with the parent peptides. We did not observe any change in the activity which means that incorporation of the DOPA moiety does not have any disturbing effect (Fig 2) to the ligand receptor interaction. These ligands are being used for the isolation of the cell surface receptors responsible for the interaction with the peptide ligands discovered in this project.

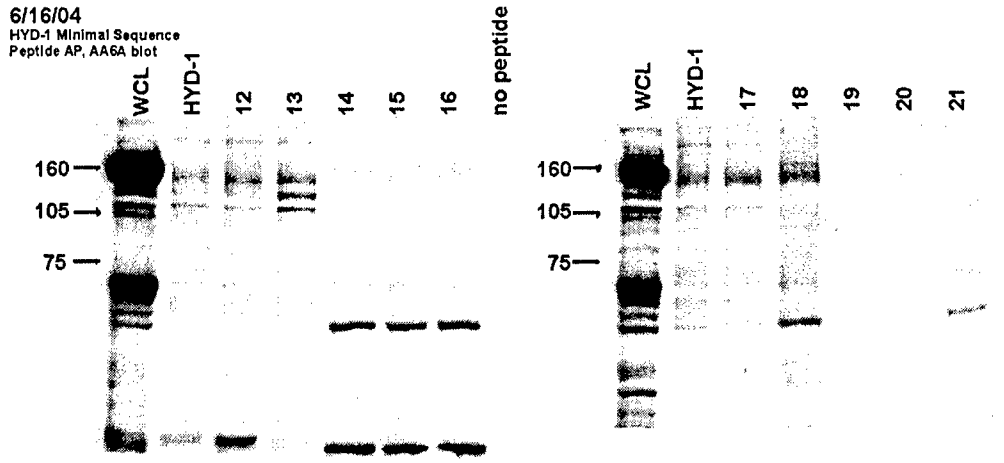

Fig 4: Western blot analysis of isolated alpha6 (MW 160 kDa)

Key Research Accomplishments

Task 1a Two combinatorial cyclic peptide libraries were synthesized with different ligand density on the exposed surface. The design of libraries was based on NMR study and molecular modeling experiment performed with previously identified peptides.

Task 1b Libraries from task 1a were screened for cell binding of prostate carcinoma cell lines DU-145, LNCap and PC3 with negative results.

Task 1a Two secondary libraries based on _kmvi_w motif were synthesized and screened.

Task 2 Monomeric and dimeric analogues of the most active peptides were synthesized an their activity was identical to the parent peptides.

Task 3a Structure activity experiments were performed with (kikmviswkg) peptide identified previously in our lab [1].

Task 3b Peptides from task 3a were tested for cell staining using flow cytometry and fluorescently labeled streptavidin. The amino acids essential for interaction of the peptide with cell surface receptors were identified.

Task 4a Libraries from task 1a were screened with other cancer cell lines (ovarian, prostate, lymphoma, lung) and compound with high affinity towards ovarian carcinomas ES-2 and SKOV-3 were identified.

Task 4a Peptide ligands based on kikmviswkg were tested with normal prostate cell lines PrSC and PrEC and they were found specific for carcinoma cell line DU-145H.

Task 5a The monomers and dimers of peptides containing DOPA were prepared and the activity was found identical if compared with parent structures.

Task 5b Truncated peptide ligands were used to isolate alpha6 integrin from the cell lysate.

Reportable Outcomes

Olulanu Aina, Jan Marik, Ruiwu Liu, Derick H. Lau, Kit S. Lam Identification of Novel Targeting Peptides for Ovarian Cancer, Cancer Research, Submitted.

Aina, O. H.; Marik, J. and Lam, K. S. Identification of Novel Peptide Ligands for Ovarian Adenocarcinoma. In 6th Joint AACR/JCA Conference, *Advances in Cancer Research*, Waikoloa Village,HI January 25-29 2004.

Conclusions

In the funding period, we synthesized several secondary combinatorial libraries based on the known ligands for DU-145H prostate cancer cell line. The libraries were screened with DU-145H cell line in order to identify more efficient ligands. The lead peptide ligands were subjected to the structure activity studies and the secondary structure of cyclic ligands were determined using NMR spectroscopy followed by molecular modeling. The research resulted in minimal sequence D amino acid heptapeptide which retains the activity of the parent specific decapeptide and the amino acids essential for the ligand receptor interaction were identified. The most active ligands were used for preparation of multimeric peptide ligands and ligands decorated with dihydroxyphenylalanine for chemical cross-linking of the ligand and receptor for further receptor isolation. The preliminary experiments shown that the peptide can be used for isolation of alpha6 integrin from the cell lysate

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